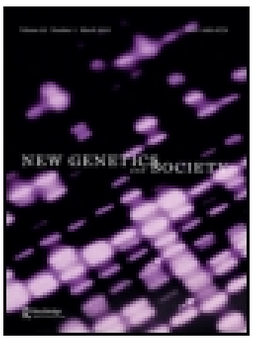
A feeling for the (micro)organism? Yeastiness, organism agnosticism and whole genome synthesis



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A feeling for the (micro)organism? Yeastiness, organism agnosticism and whole genome synthesis

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Synthetic biologists attempt to apply engineering principles to biological systems. This involves treating organisms as "chassis" – neutral frames into which synthetic constructs can be inserted, rather than living entities with distinctive features. Here we focus on a particularly charismatic organism – *Saccharomyces cerevisiae* (brewer's yeast) – and the attempt to make a synthetic version of its genome. We argue that the "personality" of the yeast and the affective relationship scientists (and others) have to it, challenges the "organism agnosticism" of synthetic biology. This leads us to ask whether synthetic biologists have straightforwardly exploitative relationships to the organisms they work on. We connect this "feeling for the (micro)organism" to the activity of engineering whole genomes, rather than discrete genetic parts. We argue that this connection is significant because we are likely to see an escalation in attempts to synthesize complete genomes in the future, including the human genome.

Keywords: synthetic biology; synthetic genomics; engineering; yeast; multispecies studies; affect

Introduction: synthetic biology, synthetic genomics and synthetic yeast

Synthetic biologists aim to engineer and control living cells, but their attempts are often thwarted by the humility-inducing complexity of the biological substrate, which tends to mutate, evolve and respond to its context (Weiss 2009). This confrontation between the audacity of engineering and the "hopeful contingencies of biology" (Davies 2011, 439) is a rich site for research in science and technology studies (STS).

Synthetic biology is a heterogeneous collection of approaches to designing and building with DNA (O'Malley *et al*. 2008), but most social scientific and philosophical work on the topic has explored the "parts-based" approach, which has been synthetic biology's posterchild since the early 2000s (Frow 2013). It is dominated by the aspiration to "make biology easy to engineer" (Endy 2008, 340) by

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constructing modular, standardized genetic parts and circuits that are inserted into recipient cells, where (in theory) they perform the synthetic biologist's specified function (O'Malley *et al*. 2008). The success of this approach is assessed in terms of how well the inserted parts and circuits produce the desired product or behavior. Recently, however, a branch of synthetic biology that aims to synthesize the complete genomes of existing organisms has been gaining ground. This could be thought of as a natural progression. As Palsson (2000) forecast nearly two decades ago: "We will move from talking about genetic engineering of single genes, to what may become known as 'genome engineering', where the whole organism is the context of the design" (1149). When "the whole organism is the context of the design" the focus shifts from the expression of an inserted genetic construct to the phenotype of the organism – its observable characteristics.

Although gene-editing technologies such as CRISPR-Cas9 are currently generating much excitement, we argue that more sociological and philosophical attention needs to be given to construction at the whole genome scale, particularly because we see these genome "writing" projects (as they are called) rapidly increasingly in number (Chari and Church 2017) – the most contentious of which sets out to synthesize the human genome.[1] Unlike the *sequencing* of whole genomes – which involves determining the order of nucleic acids – *synthesis* provides opportunities for creativity and novelty since it allows scientists and engineers to completely reimagine and re-design existing genomes. The synthetic yeast project, as the largest whole-genome synthesis project so far, is the ideal starting point for analyzing these ambitious endeavors.

In 2015, we became involved in this international effort to construct a complete yeast genome entirely from laboratory-synthesized DNA. What distinguished the synthetic yeast project from synthetic biology projects we had studied previously was that the organism itself – its distinctive qualities and features – was central. In much synthetic biology, the cell that provides the context for engineering a genetic pathway of interest often goes unmentioned or is described as a "chassis," a neutral frame into which engineered constructs can be inserted. In contrast, the yeast is prominent in the synthetic yeast project. Scientists even speak of attempting to preserve its "yeastiness" (Boeke in Urquhart 2014) in their synthetic version. This emphasis on the organism not only gave us a new perspective on synthetic biology but also showed that a connection could be made between this new phase of large-scale genome engineering and the "microbial turn" in the social sciences, in which microbes are being recognized and investigated as significant components of multispecies societies (Paxson and Helmreich 2014; Szymanski 2018a). This work draws attention to how microorganisms always exist in relation to other organisms, including humans. And yeast is not only a microbe, but a microbe with which humans have an unparalleled relationship, because of its history as a highly domesticated and uncommonly tractable organism that travels through scientific, cultural and industrial worlds. Our research on the synthetic yeast project shows the affective and conflicted relationships that the researchers

on the project have to this organism, which they both willingly "torture" (to use their term) and affectionately nurture, as will be explored in more detail below.

The synthetic yeast project centers on a microorganism that we have come to see as particularly charismatic, but we argue that yeast's distinctive features come to the fore in this project in large part because it involves engineering a whole genome rather than a discrete part or pathway. Drawing on our study of the project, we suggest that in whole-genome engineering the properties of the organism have to be taken into account in ways that do not apply in parts-based synthetic biology and that this attentiveness to the organism will become increasingly important as synthetic biologists escalate their attempts to construct the genomes of a diversity of living things.

In what follows, we start by describing the methods we used in our investigation of the synthetic yeast project. We then discuss the idea of "organism agnosticism," which is central to the engineering-driven parts-based approach to synthetic biology, but which does not describe the distinctive relationship with the organism we find in the synthetic yeast project. We briefly explain why yeast is such a scientifically and culturally significant organism, before situating the synthetic yeast project in the context of previous whole genome synthesis projects. We go on to describe the design principles that have been adopted in the synthetic yeast project and the values underlying them. We discuss the "aggressive" (Yong 2014) changes being made to the yeast genome, including the introduction of a "neochromosome" and the insertion of a mechanism to rapidly evolve the yeast on demand. We show that despite these radical changes being made to the yeast, the scientists on the project maintain an affective relationship to it. We argue that they have a "feeling for the (micro)organism," and that they sometimes describe themselves as working *with* the yeast to achieve shared goals. We then briefly turn to the synthesis of whole bacterial genomes, specifically *Mycoplasma*, to ask whether these projects also exhibit a feeling for this (very different) microorganism. We end by addressing a recent initiative to synthesize the human genome, an organism it is particularly hard to be agnostic about.

Methodology

Our ongoing social scientific study of the synthetic yeast project started in early 2015. We have visited 9 of the 11 sites participating in the project,[2] and interviewed most of the scientists directly involved, conducting 26 interviews in the UK, France, Singapore, Australia, China and the USA. We have attended conferences and meetings on the topic and participated in a week-long intensive synthetic yeast summer school in 2016, which involved practical lab work and lectures. We also spent one to two days a week at a synthetic yeast lab for 20 months from October 2015 to June 2017 and took part in weekly lab meetings. This laboratory has been involved in synthesizing three of the yeast chromosomes and has had responsibility for the "neochromosome" (discussed below). We have read widely in

synthetic biology and yeast genetics and analyzed the peer-reviewed and popular literature on the project (Calvert and Frow 2015; Szymanski 2018b).

Our social scientific work has been part of a larger scientific research grant from one of the UK Research Councils primarily focused on laboratory science. We were initially integrated into this project on the loosely specified grounds that we would engage with the "the social dimensions of the scientific work underway" as the research proposal put it, but our empirical research led us to questions about the place and significance of the organism in the synthetic yeast project that we did not initially anticipate.

Organism agnosticism

The phrase "organism agnosticism" emerged from our discussions with synthetic biologists. For example, Tasha (pseudonym)[3] describes herself as "intellectually promiscuous and organism agnostic" because instead of specializing in the engineering of one particular organism she works across plant, bacteria, yeast and mammalian cells. This idea of organism agnosticism is widespread among synthetic biologists, who often pride themselves on being generalists. They bemoan the organism specificity of current genetic techniques and maintain that their aim is to "build toolkits for genetically modifying any organism" (Gandall, Pownall, and Kahl 2017). Some synthetic biologists go so far as to state explicitly that "lack of respect for species barriers" (Voigt quoted in Hayden 2011) is a defining characteristic of their field.

The idea of organism agnosticism is closely tied to the engineering agenda of parts-based synthetic biology, in which instead of talking about an organism, synthetic biologists prefer to use "chassis" to refer to their host cell of choice.[4] The use of this word, drawn from mechanical engineering, is an explicit demonstration of the parallels synthetic biologists like to draw between their work and other engineering disciplines (de Lorenzo 2011). In the first use of "chassis" in a biological context, Canton identifies it as "a host cell that is capable of supplying the demands of an engineered system … while simultaneously insulating the engineered system from the environment" (Canton 2005, 10). An automobile chassis provides a visual reference. As in mechanical engineering, an ideal chassis should function predictably and independently of the synthetic constructs it contains (Canton and Endy 2005). The use of the word "chassis" here encourages us to think of the cell as a neutral backdrop. It removes the "organism-ness" from the organism.[5]

The term "agnostic" was coined by Thomas Henry Huxley in 1869 (Flew 2011). It means "without knowledge," originally meaning without knowledge of God. It is used colloquially to mean "not committed to or persuaded by a particular point of view" or "sceptical" (OED 2018). But synthetic biologists use the word as it is used in the computing field, in the sense of "Compatible with or available for more than one type of computer system or operating system" (OED 2018). Agnosticism in

information technology "translates to the ability of something to function without 'knowing' the underlying details of a system that it is working within" (Rouse 2016). The prospect of not needing to understand the details of a system to intervene in it is appealing to many synthetic biologists working to create a field where those who are not trained in biology – from engineers and computer scientists to do-it-yourself biologists and everyday consumers – will be able to engineer living things (Endy and Deese 2005). "Organism" is attached to "agnosticism" mainly in the context of computational tools used to manage DNA, including comparative genome browsers and bioinformatics tools.[6] The term is found in the synthetic yeast project with respect to software developed for genome design (GeneDesign and BioStudio). Although this software was made specifically for designing yeast genomes, it is described as "open source, organism-agnostic, and freely available to the public, in the expectation that the algorithms and standards it introduces will be useful to other large scale synthesis projects" (Richardson 2011, 52).

This shows an explicit connection between organism agnosticism and the software used in the synthetic yeast project. But we argue that this agnosticism does not extend to the biological organism itself. Quite the opposite in fact. What we see in this project might be called "a feeling for the (micro)organism" – a slight modification of "a feeling for the organism," the phrase Keller (1983) famously used to describe the work of Barbara McClintock, a Nobel prize-winning maize geneticist who was known for the scientific insights she developed thanks to her intimate knowledge of her research organism.

Introducing yeast

That this feeling for the microorganism is particularly apparent in the synthetic yeast project is perhaps not surprising, because humans and yeast have been working together since at least the sixth millennium BCE, when we find the first evidence of deliberate wine fermentation (McGovern, Jalabadze, and Batiuk 2017). Yeast's power of transformation through fermentation – familiar and yet still seemingly magical – has been an object of fascination for centuries (see for example Huxley 1871). This historical connection is foregrounded by researchers working on the synthetic yeast project, who often start their presentations referring to our long-standing association with yeast, showing, for example, images of ancient Sumerian pottery depicting people sharing alcoholic beverages. They also point out how yeast is essential to the production of bread, beer, sake, soy sauce, chocolate and numerous other fermented foods and drinks that many of us could not imagine living without.

It is understandable that an organism so amenable to producing food and drink was also amenable to biotechnology and genetics. Significantly, the first pure culture of *Saccharomyces cerevisiae* was isolated in 1883 in the Carlsberg Institute in Copenhagen as part of the Institute's quest to create reliable beers (Bud 1998).

There is an intriguing history of how yeast became central to the early days of microbiology and then developed as a key model organism for contemporary biology, partially thanks to its unusually flexible genome, which has been modified and selected through the twentieth century to yield yeast strains especially well-suited to molecular genetics (Langer 2016).

Saccharomyces cerevisiae is highly domesticated, to the extent that it is found to a much greater extent in fermenters and laboratories than in the wild (Gallone, Steensels, and Prahl 2016). And the scientists on the synthetic yeast project regularly use the language of domestication, often talking about dogs and cows when describing their relationship to yeast. They say that like these animals, yeast has coevolved with us, and is already a product of human intervention, interaction and collaboration. This means that the synthetic yeast project does not start with a "natural," wild yeast, which is then engineered and made "unnatural." While the phrase "wild-type" is employed to describe the yeast that serves as the template for engineering in the synthetic yeast project, this "wild-type" is, in fact, a highly domesticated laboratory strain.

In addition to its history of domestication, yeast has other features that make it a good organism for synthetic biology. It grows faster than many other laboratory organisms, enabling hypotheses to be tested more quickly (Beam 2009). It is familiar to non-scientists and "generally recognized as safe" (US Department of Health & Human Services 2013). In terms of genetics, *Saccharomyces cerevisiae* is described by one of the scientists on the project as "The greatest organism on earth" (Vic at Sc2.0 summer school), because its genes are nicely laid out on the different chromosomes, rather than overlapping as they often are in bacteria, making it relatively easy to engineer. Yeast is a eukaryote, like plants and animals, but has far less epigenetic complexity than most other eukaryotes. And because of its status as a model organism, there are a huge number of genetic tools for working on yeast, including the *Saccharomyces* Genome Database (SGD), which collates yeast information resources by and for a large international community.[7] Additionally, *S. cerevisiae* was the first eukaryotic genome to be sequenced, and the annotations associated with the yeast genome are very well curated and understood (Botstein and Fink 2011).[8]

Furthermore, yeast are regularly referred to as charismatic and friendly. One of the scientists on the synthetic yeast project says of this organism:

> We know a lot about it, we love it, we test all molecular biology and biochemical tools on it, it's very familiar, it's a "go to", it's a good buddy, it's such a good buddy we make mushy toys of it; it's quite the friend. And what do you do when you have such a good friend for so long? Do you mess with its genome even more? Because that's what we did. (Richardson 2017)

This brings us to the synthetic yeast project.

The synthetic yeast project

The synthetic yeast project aims to re-imagine and re-design the complete genome of this familiar organism. The modified yeast has been named *Saccharomyces cerevisiae* 2.0, or Sc2.0 for short, following the software naming convention common in synthetic biology. This name encourages us to think of the yeast genome as a computer program, which can be re-coded at will (Szymanski 2018b).[9]

Yeast is not the first whole-genome synthesis project, although it is the largest so far. The earliest examples are usually identified as the compete synthesis of two viral genomes: the poliovirus in 2002 (Cello, Paul, and Wimmer 2002), and the bacteriophage *phi*-X174 in 2003 (Smith *et al*. 2003). The first bacterial genome to be synthesized (by the J. Craig Venter Institute) was *Mycoplasma genitalium* in 2008, which has one of the smallest known bacterial genomes (Gibson 2008). This was followed by the slightly larger and more laboratorially-tractable genome of *Mycoplasma mycoides* in 2010 (Gibson 2010), which received front-page coverage around the world, since it was the first time a completely synthetic bacterial genome was successfully replicated by a recipient cell. In 2016 a minimized version of *Mycoplasma mycoides* was synthesized by the same group (Hutchinson *et al*. 2016), resulting in "the smallest self-replicating organism known" (Nature Biotechnology 2016, 673). We return to these *Mycoplasma* projects below. Also in 2016, the complete genome of a much larger bacteria, *E. coli*, was synthesized with systematic changes made to codons (DNA base-pair triplets that encode amino acids) across the whole genome (Perkel 2017). The yeast genome is an order of magnitude larger than these bacterial genomes (Jovicevic, Blount, and Ellis 2014).

Because of the size of the genome, the Sc2.0 project is an international effort, with chromosome synthesis distributed across a consortium of nine laboratories in the UK, the USA, Australia, Singapore, and China, with another two laboratories in France and Germany analyzing the completed synthetic chromosomes.[10] Bacteria typically have a single circular chromosome, but eukaryotic genomes are divided into multiple chromosomes – 16 for yeast – making it reasonably straightforward for chromosomes and labor to be distributed around the consortium. Across this large and geographically diverse group, the scientists involved bring with them a range of different interests and expertise. Some are self-defined synthetic biologists who have been active in parts-based approaches to the field and see the Sc2.0 project as a proof-of-principle of large-scale synthetic biology.[11] Others are experts in yeast genetics and want to use constructive techniques to find out more about their favorite organism.

Constructing synthetic yeast

The scientists working on the Sc2.0 project often describe it as an attempt to "refactor" the entire yeast genome (Richardson *et al*. 2017). The term "refactoring" comes from computer engineering and refers to the process of rationalizing and tidying up computer software by removing redundancies and inconsistencies

(Fowler *et al*. 1999). Synthetic biologists have attempted to order and simplify many different types of genetic sequence in a similar manner, including viral genomes (Chan, Kosuri, and Endy 2005) and nitrogen fixing genes (Temme, Zhaob, and Voigt 2012). The use of the term "refactoring" again encourages a parallel between genomes and computer software, and downplays differences between organisms. Refactoring necessarily involves design choices with respect to what constitutes a "tidier" sequence, and in this particular project many consequential decisions have been made.

During a conference presentation, the PI of the Sc2.0 project described how when he and his first collaborator decided to build a yeast genome they felt that possibilities were infinite and they did not know which design decisions to make. Seeking help, they asked the community of yeast geneticists for suggestions. As he recounts: "the silence was deafening. Most people thought we were totally and utterly insane" (Boeke 2016). Left to their own devices they decided to "design a yeast that can teach us biology" – to make a genome that would help further biological understanding. The design involved "a laundry list of arbitrary decisions" (Boeke 2016) and took a year to finalize.

The centrality of design to this project, and to synthetic biology in general, makes it a rich topic for social scientific investigation. The capacity for design provided by synthesis not only allows for creativity and novelty, it also involves choices and expectations about future use. In other words, design always involves values – it is about making the designed entity "better" for some anticipated purpose. In this way we see ideas about what would be most interesting and useful in the future being built into the design of this organism. What appears to be a merely technical "refactoring" exercise is imbued with values. We describe these design choices in some detail because yeast is the first eukaryotic genome to be synthesized, and what is deemed to be successful in yeast is likely to be applied to the synthesis of other genomes in the future.

The final design for the Sc2.0 project was guided by three overriding principles: to maintain the fitness of the yeast, to maintain its genomic stability, and to increase its genetic flexibility. There is an interesting inconsistency in these design principles: while the second and third refer to properties of the genetic material, the first refers to the fitness of the organism overall. Tellingly, this first principle is sometimes expressed by the phrase "do no harm to the yeast." This is significant for our argument because although the design, engineering and construction in this project are all focused on the DNA, the scientists judge the success of their genetic changes by assessing the fitness of the whole organism. And the way in which they assess its fitness is phenotypically – usually by looking at whether the yeast grows as rapidly as would be expected on agar plates or in liquid-media cultures. This means that the whole organism, its behavior and its phenotype, is the focus of the majority of the laboratory work. This focus, we argue, represents much of what is distinctive about whole genome work and is responsible for the continual surfacing of the organism.

Many changes are being made to the genome to further the design principles, and these changes are often described as "aggressive," to the extent that some ask whether they might even result in the creation of a new species.[12] Such aggressiveness is represented by a cartoon depiction of the synthetic yeast project from the *Economist* from 2014 to accompany an article on the first chromosome synthesized by the project. In this cartoon, four scientists are shown cutting, sawing, and even electrocuting the genome remotely via a huge screen above their heads.[13]

The changes that have been made include removing introns (non-coding sections of DNA that are found in the midst of coding sequences), shortening telomeres (repetitive sequences of DNA at the end of each chromosome), and eliminating all instances of one codon to free it up for some other future use.[14] One aim of the redesign is to remove repetitive regions that are considered redundant, and the final Sc2.0 genome will be approximately 8% shorter than the original (Pretorius and Boeke 2018). But the most radical change being made to the genome, as part of the attempt to increase its genetic flexibility, is the introduction of so-called "SCRaMbLE" sites,[15] that make it possible to induce "superfast evolution on demand" (Clive at Sc2.0 summer school). These sites allow large-scale genome rearrangements – deletions, inversions, transpositions, and duplications – that would never be seen in nature. In this way, SCRaMbLE gives rise to completely new genome sequences and opens up experimental trajectories that would not be possible otherwise.[16]

Another notable element of the design is the "neochromosome," the construction of which we followed closely in the laboratory. Of all the chromosomes, this is the one most explicitly influenced by design decisions, because it is being built without a wild type comparator. Rather, it has been designed and constructed from scratch by the scientists working on the project. The reason for building the neochromosome is to increase the stability of the genome overall by putting the genetic elements perceived to be most unstable – the transfer RNAs (tRNAs) – in one place (Walker 2017). The neochromosome's design audaciously transgresses organismal boundaries by incorporating DNA from nine different yeast species, with only 10% of the sequence from *S. cerevisiae*. This eclectic range of species is used because the scientists are designing the neochromosome to operate independently of the rest of the genome.[17]

The aggressiveness of the Sc2.0 design is apparent in all these modifications. The design initially appeared to be so radical that the researchers were not sure it was going to succeed since "nobody had ever done this depth of damage to the genome" (Ken interview). In fact, the researchers regularly use the language of "torture" when describing how extensively they are changing the yeast. For example, in a presentation during the early days of the project, the PI said that one of the most pressing questions they wanted to address was "How much can we torture them and still have a living cell?" (Boeke 2011). And he is more recently quoted as saying "It is amazing how much torture the yeast genome can take and still be happy and healthy" (Boeke in Holmes 2017).[18]

As this last quotation indicates, the researchers have been continually surprised at the tolerance of the yeast. They conclude that it is "highly amenable to and tolerant of genetic manipulation" (Mitchell 2015, 6620) because the design changes have had very minimal effects on fitness. More colloquially, it's "an easy-going organism" (Molly interview). This shows that in the project there is regular reference to the particular features of the organism; what we might call its "personality." And there is a deliberate attempt to preserve the personality of yeast in its synthetic form. Even with the radical changes that are being made to the genome, there is talk of how its "yeastiness" must be maintained (Boeke in Urquhart 2014).

Consistent with this objective, the researchers often stress that the overall aim of the project is to create a "happy, healthy yeast" (Boeke in Duhaime-Ross 2014). When we probed them further we found that both health and happiness are measured in terms of the phenotype of the whole organism, with how the yeast look (Steve interview). The scientists are attentive to whether the white spots of yeast colonies that grow on petri-dishes look normal rather than "sick," whether their size is normal, what variation there is between colonies, and whether the cells they see under the microscope seem ready to divide (Kris and Mary interviews).

A feeling for the microorganism?

We may be tempted to be skeptical of this talk of keeping the yeast "happy" in the context of the extensive levels of intervention, manipulation, "aggression" and even "torture" in the project. At first glance, we seem to be observing something very different from the "feeling for the organism" that Evelyn Fox Keller uses to describe Barbara McClintock's work on maize. McClintock was notable for her painstaking, almost reverential attention to the biological. Many people would see synthetic biology as the complete antithesis of this approach; where McClintock sought to understand the biological world around her, synthetic biologists typically aim to overwrite that world with their own designs. But the seemingly paradoxical relationship that the scientists on the project seemed to have to their organism suggests a more nuanced interpretation. On the basis of our observations, and drawing on multispecies work that emphasizes the active participation of microbes in human endeavors (van Dooren, Kirksey, and Münster 2016), we decided to tackle this by asking our interviewees: are you working *on* or *with* the yeast?

Some scientists clearly think of themselves as working on the yeast, saying, for example, "At the moment I think of it more as a tool. We're building ourselves this awesome tool" (Nat interview). Or maintaining: "I think yeast has much higher value as a platform than as an organism to be investigated … you can think of it as a very dedicated machine" (Bill interview). But this instrumental attitude is not found consistently. For example, a senior scientist says "it feels like a partner" (Ken interview), but in the same breath adds that "it's a very interesting

bag of DNA." The yeast seems to lend itself to both these descriptions simultaneously.

Others unhesitating describe themselves as working *with* the yeast. For example, one said that the yeast would be excited by the project, because of the scientific knowledge it would generate, and the potential applications that could result (Molly interview). She argued that since yeast is already domesticated, its instrumentalization in the synthetic yeast project is no different than its involvement in making bread or beer. Another scientist expressed a similar view that the potential of the yeast had not yet been unlocked and that microbial engineering is "a powerful way for us to harness what biology already gave us" (Nat interview). In this light, it is telling that some project members like to describe themselves as "yeast whisperers" (Pretorius and Boeke 2018, 10). A whisperer is known for their rapport with animals and the rejection of abusive training methods (Birke 2007).

This idea of working *with* the organism towards a shared goal is a feature of our interviews and observations partially because of the yeast's facility for homologous recombination, i.e. to "take two different pieces of DNA that have original homology and splice them together" (Larry interview). Homologous recombination is essential to building a synthetic genome, making the yeast's abilities crucially important to the project because "we're using yeast to do parts of the synthesis and assembly steps" (Larry interview).[19] In this way, the work of genetic construction is shared between the synthetic biologist and the organism. This ability of the yeast is a major component of what is often referred to as "the awesome power of yeast genetics".[20] In fact, homologous recombination and collaboration are explicitly linked by one of the scientists on the project who explains: "because it does take up DNA so well so we're working *in collaboration* with our organism essentially, in the way that we're able to integrate our DNA" (Mitchell 2017, emphasis added).

What we find striking here is the ambivalent, multiple – maybe even contradictory – relationships that the scientists have to the yeast, which are simultaneously regarded as tools and partners, machines and collaborators. Other STS work finds similar apparent contractions in the relationships between scientists and microorganisms. For example, McLeod, Nerlich, and Mohr (2017), in another study of synthetic biology, describe how bacteria are conceptualized both as "machines or tools (useful for creating wealth) and as personified agents (that deserve care and respect)" (6). And Lorimer's (2017) investigation of the hookworm – a microbe sometimes found in the human gut – shows that it can be simultaneously regarded as a dangerous parasite and an "old friend" (6) essential for proper immune functioning. We are not maintaining that these seemingly paradoxical relationships are only found between scientists and microorganisms, of course. Myers (2015) notes "that peculiar mixture of love and violence that has long conditioned experimental work in the life sciences" (59). And others have shown that multifaceted and complex relationships among researchers and their organisms of choice are not

unusual (e.g. Kirk 2014; Kelly and Lezaun 2014). Some argue that such affective labor is inseparable from scientific practice (Fitzgerald 2013). But our point is that yeast, because it is a microorganism with which we have had such long and close associations, brings these relations to the fore even in an engineering context where we might not expect them.

The relationship the scientists on the project have to the organism is complex, and far from merely instrumental. There is an excitement about the potential of the yeast, which is celebrated because of its adaptability and manipulability, and because of the things that can be done in collaboration with it. These observations resonate with Keller's (1983) description of the pleasure McClintock has in the flexibility of organisms, and their capacities to meet their needs "in ways that never cease to surprise us" (199). Of course, the scientists discussed here are very unlike McClintock in that they do not prioritize patient, detached attentiveness to their organism, but the synthetic yeast project shows that intervening and manipulating can be combined with appreciation, and that engineering does not imply a straightforwardly exploitative relationship to living things.

A feeling for *Mycoplasma?*

We have argued that these more complex and overt relationships to the organism surface when whole genomes are being constructed. While in parts-based approaches the intention is that "orthogonal" engineered entities will not interact with the chassis that hosts them,[21] constructing whole genomes differs because "we are building everything and therefore everything we build needs to integrate with everything" (Molly interview). If there is a significant difference between engineering parts and engineering wholes, and if the latter challenges the organism agnosticism of synthetic biology, then this same "feeling for the (micro)organism" should apply to other attempts to build whole genomes, which is why we briefly turn to the J Craig Venter Institutes' *Mycoplasma* projects.

In contrast to Sc2.0, these projects were all driven by the objective of identifying the minimal genome necessary for life, which is why they started with *Mycoplasma,* the simplest cells capable of autonomous growth (Hutchinson *et al*. 2016). The Venter Institute's first two *Mycoplasma* projects in 2008 and 2010 created synthetic versions of naturally existing genomes (with some added "watermarks" in the 2010 version), but the 2016 project aimed to do something more radical: to minimize this already-tiny genome by removing all non-essential genes from the design (Hutchinson *et al*. 2016). Working out what constituted an "essential" gene proved to be more complex than anticipated, however, and the conclusion that the researchers eventually drew was that there was no one minimal genome, the interesting consequences of which are beyond the scope of this paper.

We have not conducted a laboratory study of the Venter Institute's *Mycoplasma* work, so we do not have direct evidence that the scientists working on these projects had a feeling for their organism. Although preserving the "personality" of this

bacteria is not a central objective of these projects, we nonetheless see regular reference to the distinctive characteristics of *Mycoplasma*. They are delicate, with no cell walls, and need to be treated far more gently than yeast in laboratory procedures.[22] And the phenotype of the organism is prominent: the 2016 paper has microscopic images of strange and atypical "filamentous and large-vesicle morphotypes" (Hutchinson *et al*. 2016, 10) to show how the synthetic cell – with the smallest known genome in existence – instantiated itself in physical form.

Mycoplasma may not, on first encounter, seem as charismatic as yeast. These tiny, delicate, opportunistic pathogens are not as familiar and adaptable as *Saccharomyces cerevisiae*; they do not have a similar history of domestication, nor the wealth of connections to users outside scientific institutions (such as bakers and brewers). But we should not be surprised that different microbes have different personalities. On the contrary, this is what we should expect of this hugely diverse collection of living things. Although *Mycoplasma* are very different from yeast, we see scientists engaging with the specificity and distinctiveness of this organism, and this gives further support to our argument that when scientists engineer at the level of the whole genome they are compelled to engage with the whole organism.

We end with a final example of the ways in which whole-genome engineering challenges organism agnosticism, which arose unexpectedly from our study of synthetic yeast.

A feeling for the human?

Once the success of the Sc2.0 project seemed likely, discussion started on the question of which genome should be synthesized next. Following the path of DNA sequencing ("reading"), the synthesis of another genome ("writing") was assumed to be a natural next step. Arguments were made in favor of synthesizing a familiar model organism like the worm *Caenorhabditis elegans* or the fruit fly *Drosophila melanogaster*,[23] but the leaders of the synthetic yeast consortium, in collaboration with other prominent figures in synthetic biology, chose the human (Boeke *et al*. 2016). They planned to apply tools and principles used in the synthetic yeast project, including SCRaMbLE (and its capacity for massive unprecedented genomic rearrangement) to the human genome (Boeke *et al*. 2017).

The first dedicated "Human Genome Project-write" meeting took place at Harvard in 2,016 and provoked immediate negative reactions from bioethicists, synthetic biologists, and others, who expressed concerns about potential ethical ramifications (including human cloning and germ-line modification) and the lack of prior public consultation (e.g. Endy and Zoloth 2016). The idea of *Homo sapiens* 2.0 immediately elicits a response, perhaps because is hard to be organism agnostic when we are talking about our own species.

The focus on this particularly prominent organism lent added significance to questions we have already raised in relation to the synthetic yeast project, such as: which design decisions would be made in synthesizing a human genome

(Endy and Zoloth 2016)? Which values would be built into those designs? And who would decide? Closely linked concerns related to the funding, control and coordination of the research and its applications (Krieger 2016), and whose interests the HGP-write project would serve (Yong 2017).

Technical objections were also made. Some observed that the challenges of synthesizing human genomes are much greater than those for synthesizing yeast, largely because of the sheer length of the human genome, which has three billion base pairs compared to yeast's 11 million. They maintained that "The huge effort and money spent on creating a full complement of 23 synthetic human chromosomes may be a matter of diminishing returns" (Nature Biotechnology 2016, 673). Others warned that the success of the Sc2.0 project should not necessarily lead to the conclusion that other genome sequencing projects would be equally productive. Richardson (2017) argued that the Sc2.0 project has been successful because "yeast loves us back." She suggested that before taking on the synthesis of another genome it is advisable to "step 50 feet back," and ask: is it as familiar and friendly to us as yeast?

Since its first meeting this project has changed strategy. Re-labelled "Genome Project-write," the objective has shifted to synthesizing the genomes of a range of different organisms. Various plant and animal genomes, including cephalopods, are being considered (Boeke *et al*. 2017), in part to diffuse concerns over focusing on a synthetic human genome. The project may have broadened, but its existence demonstrates the growing interest in whole-genome synthesis, and the increasing emphasis on the organism being synthesized.

Conclusions

We have reflected this growing interest through our study of Sc2.0, the largest whole-genome synthesis project to date. We have identified a "feeling for the (micro)organism" in this project, brought into focus by the special relationship between humans and yeast. We have argued that this challenges the engineering ideal of organism agnosticism, prominent in parts-based synthetic biology. Our research on synthetic yeast also shows that these synthetic biologists do not have straightforwardly exploitative relationships to the organisms they manipulate. Although the scientists in Sc2.0 have made "aggressive" changes to the genome, they are keen to preserve the organism's "yeastiness" by keeping it healthy and happy. They are excited by the potential of the organism, which they work *with* in an intellectually and affectively entangled manner. This is not to deny that they often engage with the organism in instrumental ways. But what we have observed is their multiple, sometimes conflicting, relationships to the yeast. We argue this recognition of multiplicity enriches the discussion of synthetic biology.

We have drawn attention to the distinctive characteristics of *Saccharomyces cerevisiae*: its long history of domestication, industrial utility, manipulability, and the multitude of genetic tools available to work on it. But we have suggested that it is

because the scientists are engineering the whole genome that they engage with the specific features of the organism. Our brief discussions of the Venter Institute's genome synthesis work and the Genome Project-write suggest that our conclusions extend more broadly. Engaging with the whole organism seems to drive synthetic biology to become more *biological*; the organism assumes a character and significance that makes it difficult to treat as merely another entity to be engineered. In whole genome engineering, the "hopeful contingencies of biology" (Davies 2011, 439) reassert themselves.

As the synthetic yeast project shows, these biological propensities and capacities can be harnessed to create something new. Whole-genome synthesis provides unprecedented opportunities for re-imagining and re-designing existing genomes, and synthetic biologists are starting to ask questions like that posed by Voigt (2017): "Now that we have the ability to synthesize anything, what do we build, what do we design with that capacity?" As whole-genome projects proliferate, and value-laden design decisions are made, it is important that a diverse range of people become involved these discussions. Whole-genome synthesis not only compels us to reconsider our relationships to existing organisms, it also challenges us to engage with what they may become.

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Notes

1. For the "GP-write" project see http://engineeringbiologycenter.org/
2. The only two sites that we have not visited are Tianjin, China and EMBL, Germany.
3. Pseudonyms are used for all interviewees, unless we cite a public presentation.
4. A term also adopted by the synthetic yeast project, see Dymond and Boeke (2012).
5. In synthetic biology meetings we have observed microbiologists squirm and object when hearing "chassis" applied to their favorite organism.
6. See for example http://www.pastelbioscience.co.uk/technologies/bioinformatics.html
7. See https://www.yeastgenome.org/

8. *S. cerevisiae* is an archetypal model organism in both technical and social terms (Ankeny and Leonelli 2011). But in this project it is not being used as a model organism in the sense of being used to learn about general biological principles. However, the project is starting to be referred to as a model of *practice* – a model of how to build a synthetic genome.
9. See McLeod and Nerlich (2017) for a wider discussion of computational, mechanical and reading/writing metaphors in synthetic biology.
10. See http://syntheticyeast.org/collaborators/
11. For some, their long-term goal is to "eventually realize completely modular genomes that are built-to-design from standard parts" (Ellis 2019, 9).
12. Babcock (2019) discusses this question but concludes that synthetic genomes are part of the same genetic lineage as their non-synthetic counterparts.
13. See https://www.economist.com/science-and-technology/2014/03/27/diy-chromosomes
14. The stop codon TAG has been replaced with the codon TAA to make it possible to use TAG for an unnatural amino acid in the future.
15. SCRaMbLE stands for "Synthetic Chromosome Rearrangement and Modification by Lox-P mediated Evolution" (Dymond and Boeke 2012).
16. See Szymanski and Calvert (2018) for discussion of the role of the yeast in SCRaMbLE.
17. Even with this additional chromosome, the final synthetic genome will have sixteen chromosomes, like the wild type yeast, because two of the smallest chromosomes (I and III) will be combined.
18. Merchant (1980) traces back to Frances Bacon the metaphor of torture as a means of extracting truth from nature.
19. Homologous recombination is so essential to the work involved building a synthetic genome that many speculate that future synthetic genomes of other organisms will need to initially be assembled in yeast.
20. This is a phrase that is used regularly by yeast scientists. It even has its own hashtag: #APOYG.
21. Although in practice this is rarely the case, and much work in synthetic biology is directed towards curtailing those interactions (see Borkowski et al. 2016).
22. Based on fieldnotes from the synthetic yeast summer school in 2016, where one of us was paired with a Mycoplasma expert.
23. Fieldnotes from the "4th Annual Sc2.0 and Synthetic Genomes Conference" 16-17th July 2015, New York.

References

Ankeny, R., and S. Leonelli. 2011. "What's So Special About Model Organisms?" *Studies in History and Philosophy of Science Part A* 42: 313–323.

Babcock, G. 2019. "Are Synthetic Genomes Parts of a Genetic Lineage?" *The British Journal for the Philosophy of Science*, Doi:10.1093/bjps/axz046. Online first.

Beam, C. 2009. "The Rise of Yeast." *Slate*, May 6 2009. Online at: https://slate.com/news-and-politics/2009/05/why-do-scientists-always-use-yeast-in-their-experiments.html.

Birke, L. 2007. "'Learning to Speak Horse': The Culture of Natural Horsemanship." *Society and Animals* 15: 217–239.

Boeke, J. 2011. "The Synthetic Yeast Project." Presentation at the Fifth International Meeting on Synthetic Biology, Stanford University, USA, June 15–17 2011.

Boeke, J. 2016. "Overview of the Sc2.0 Project." Presentation at the Fifth International Sc2.0 and Synthetic Genomes meeting, Dynamic Earth, Edinburgh, July 8–9 2016.

Boeke, J., G. Church, A. Hessel, and N. Kelley. 2017." GP-write: A Grand Challenge Using DNA Synthesis, Gene Editing and Other Technologies to Understand, Engineer and Test Living

Systems May 9–10, 2017". http://engineeringbiologycenter.org/wp-content/uploads/2017/10/GP-write-2017-summary.pdf.

Boeke, J., G. Church, A. Hessel, N. Kelley, A. Arkin, Y. Cai, Carlson R., Chakravarti A., Cornish V. W., Holt L., Yang L., et al. 2016. "The Genome Project-Write." *Science* 353: 126–127.

Boeke, J., in J. Urquhart. 2014. "Synthetic Yeast Chromosome is Fully Functional." *Royal Society of Chemistry* (27 March 2014). https://www.chemistryworld.com/news/synthetic-yeast-chromosome-is-fully-functional/7222.article.

Borkowski, O., F. Ceroni, G.-B. Stan, and T. Ellis. 2016. "Overloaded and Stressed: Whole-Cell Considerations for Bacterial Synthetic Biology." *Current Opinion in Microbiology* 33: 123–130.

Botstein, D., and G. Fink. 2011. "Yeast: An Experimental Organism for 21st Century Biology." *Genetics* 189 (3): 695–704.

Bud, R. 1998. "Molecular Biology and The Long-Term History of Biotechnology." In *Private Science: Biotechnology and the Rise of the Molecular Sciences*, edited by A. Thackray, 3–19. Philadelphia: University of Pennsylvania Press.

Calvert, J., and E. Frow. 2015. "The Synthetic Yeast Project as a Topic for Social Scientific Investigation." *Macquarie Law Review* 15: 27–37.

Canton, B. 2005. "Engineering the Interface Between Cellular Chassis and Integrated Biological Systems." Thesis proposal. http://dspace.mit.edu/bitstream/handle/1721.1/19813/BC.ThesisProposal.pdf?sequence=1.

Canton, B., and D. Endy. 2005. "Engineering the interface Between Cellular Chassis and Integrated Biological Systems." Poster presented at the sixth international conference on systems biology, Boston, MA, October 19–24 2005. http://dspace.mit.edu/bitstream/handle/1721.1/29802/BC.ICSB05.pdf?sequence¼1.

Cello, J., A. Paul, and E. Wimmer. 2002. "Chemical Synthesis of Poliovirus cDNA: Generation of Infectious Virus in the Absence of Natural Template." *Science* 297 (5583): 1016–1018.

Chan, L., S. Kosuri, and D. Endy. 2005. "Refactoring Bacteriophage T7." *Molecular Systems Biology*. Article number: 2005.0018. doi: 10.1038/msb4100025

Chari, R., and G. Church. 2017. "Beyond Editing to Writing Large Genomes." *Nature Reviews Genetics* 18 (12): 749–760.

Davies, D. 2011. "Playing Dice with Mice: Building Experimental Futures in Singapore." *New Genetics and Society* 30 (4): 433–441.

de Lorenzo, V. 2011. "Beware of Metaphors: Chasses and Orthogonality in Synthetic Biology." *Bioengineered Bugs* 2 (1): 3–7.

Duhaime-Ross, A. 2014. "Scientists Just Took a Major Step Toward Making Life From Scratch." (27 March 2014) *The Verge*. http://www.theverge.com/2014/3/27/5553044/first-functional-eukaryoticchromosome.

Dymond, J., and J. Boeke. 2012. "The *Saccharomyces Cerevisiae* SCRaMbLE System and Genome Minimization." *Bioengineering Bugs* 3 (3): 168–171.

Ellis, T. 2019. "What is Synthetic Genomics Anyway?" *The Biochemist* 41 (3): 6–9.

Endy, D. 2008. "Synthetic Biology: Can We Make Biology Easy to Engineer?" *Industrial Biotechnology* 4 (4): 340–351.

Endy, D., and I. Deese. 2005. "Adventures in Synthetic Biology." *Nature* 438: 449–453.

Endy, D., and L. Zoloth. 2016. "Should We Synthesise a Human Genome?" *Cosmos Magazine*. May 12 2016. https://cosmosmagazine.com/society/should-we-synthesise-a-human-genome.

Fitzgerald, D. 2013. "'The Affective Labour of Autism Neuroscience: Entangling Emotions, Thoughts and Feelings in a Scientific Research Practice.'." *Subjectivity* 6: 131–152.

Flew, F. 2011. Agnosticism." *Encyclopædia Britannica*. Retrieved December 15, 2011. https://www.britannica.com/topic/agnosticism.

Fowler, M., K. Beck, J. Brant, W. Opdyke, and D. Roberts. 1999. *Refactoring: Improving the Design of Existing Code*. Boston, MA: Addison-Wesley Professional.

Frow, E. 2013. "Making Big Promises Come True? Articulating and Realizing Value in Synthetic Biology." *BioSocieties* 8: 432–448.

Gallone, B., J. Steensels, T. Prahl, L. Soriaga, V. Saels, B. Herrera-Malaver, K. J. Verstrepen, et al. 2016. "Domestication and Divergence of *Saccharomyces Cerevisiae* Beer Yeasts." *Cell* 166: 1397–1410.

Gandall, K., S. Pownall, and L. Kahl. 2017. "Bionet 10k Genes Project Documentation v1.0." https://www.reddit.com/r/bionet/comments/7bo7gz/bionet_10k_genes_project_documentation_v10/.

Gibson, D., G. A. Benders, C. Andrews-Pfannkoch, E. A. Denisova, H. Baden-Tillson, J. Zaveri, H. O. Smith, et al. 2008. "Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma Genitalium* Genome." *Science* 319 (5867): 1215–1220.

Gibson, D., J. I. Glass, C. Lartigue, V. N. Noskov, R.-Y. Chuang, M. A. Algire, G. A. Benders, J. C. Venter, et al. 2010. "Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome." *Science* 329 (5987): 52–56.

Hayden, E. 2011. "Synthetic Biology: Parlor trick, or Proof of Concept?" *The Last Word On Nothing Blog* 20 June 2011. http://www.lastwordonnothing.com/2011/06/20/synthetic-biology-parlor-trick-or-proof-of-concept/.

Holmes, B. 2017. "Five Designer Chromosomes Bring Synthetic Life a Step Closer." *New Scientist* 9 March 2017. https://www.newscientist.com/article/2124052-five-designer-chromosomes-bring-synthetic-life-a-step-closer/.

Hutchison III, C. A., R. Y. Chuang, V. N. Noskov, N. Assad-Garcia, T. J. Deerinck, M. H. Ellisman, J. Gill, et al. 2016. "Design and Synthesis of a Minimal Bacterial Genome." *Science* 351 (6280): 1414.

Huxley, T. H. 1871. "Yeast." In *Collected Essays*, edited by T. H. Huxley, 110–136. Cambridge: Cambridge University Press.

Jovicevic, D., B. A. Blount, and T. Ellis. 2014. "Total Synthesis of a Eukaryotic Chromosome: Redesigning and SCRaMbLE-ing Yeast." *BioEssays* 36: 855–860.

Keller, E. F. 1983. *A Feeling for the Organism: The Life and Work of Barbara McClintock*. San Francisco: WH Freeman and Co.

Kelly, A., and J. Lezaun. 2014. "Urban Mosquitoes, Situational Publics, and the Pursuit of Interspecies Separation in Dar es Salaam." *American Ethnologist* 41 (2): 368–383.

Kirk, R. 2014. "In Dogs We Trust? Intersubjectivity, Response-Able Relations, and the Making of Mine Detector Dogs." *Journal of the History of the Behavioral Sciences* 50 (1): 1–36.

Krieger, L. 2016. "Critics attack Harvard's Secret Meeting on Human Genome Synthesis." *East Bay Times*. August 15, 2016. https://www.eastbaytimes.com/2016/05/13/critics-attack-harvards-secret-meeting-on-human-genome-synthesis/.

Langer, E. 2016. *Molecular Ferment: The Rise and Proliferation of Yeast Model Organism Research*. PhD Dissertation, University of California, San Francisco. https://search.proquest.com/openview/90c3137d0d9a9ad0f46158a7697bf6dc/1?pq-origsite=gscholar&cbl=18750&diss=y.

Lorimer, J. 2017. "Parasites, Ghosts and Mutualists: A Relational Geography of Microbes for Global Health." *Transactions of the Institute of British Geographers* 42 (4): 544–558.

McGovern, P., M. Jalabadze, S. Batiuk, M. P. Callahan, K. E. Smith, G. R. Hall, D. Lordkipanidze, et al. 2017. "Early Neolithic Wine of Georgia in the South Caucasus." *Proceedings of the National Academy of Sciences* 114 (48): E10309–E10318.

McLeod, C., and B. Nerlich. 2017. "Synthetic Biology, Metaphors and Responsibility." *Life Sciences, Society and Policy* 13 (1): 13. doi:10.1186/s40504-017-0061-y.

McLeod, C., B. Nerlich, and A. Mohr. 2017. "Working with Bacteria and Putting Bacteria to Work: The Biopolitics of Synthetic Biology for Energy." *Energy Research & Social Science* 30: 35–42.

Merchant, C. 1980. "The Scientific Revolution and *The Death of Nature*." *Isis* 97: 513–533.

Mitchell, L., J. Chuang, N. Agmon, C. Khunsriraksakul, N. A. Phillips, Y. Cai, J. D. Boeke, et al. 2015. "Versatile Genetic Assembly System (VEGAS) to Assemble Pathways for Expression in *S. Cerevisiae*." *Nucleic Acids Research* 43: 6620–6630.

Mitchell, L. 2017. "How we Build and De-bug Sc2.0 Chromosomes." Presentation at SB7: The Seventh International Meeting on Synthetic Biology, National University of Singapore, Singapore, 13th-16th June 2017.

Myers, N. 2015. *Rendering Life Molecular*. Durham, NC: Duke University Press.

Nature Biotechnology. 2016. "Editorial: Rewriting our Future." *Nature Biotechnology* 34 (7): 673.

OED. 2018. "Agnostic, *n*. and *adj*." *OED Online*. December 2018. Oxford University Press. http://www.oed.com/view/Entry/4073.

O'Malley, M., A. Powell, J. Davies, and J. Calvert. 2008. "Knowledge-Making Distinctions in Synthetic Biology." *BioEssays* 30 (1): 57–65.

Palsson, B. 2000. "The Challenges of *in Silico* Biology." *Nature Biotechnology* 18: 1147–1150.

Paxson, H., and S. Helmreich. 2014. "The Perils and Promises of Microbial Abundance: Novel Natures and Model Ecosystems, From Artisanal Cheese to Alien Seas." *Social Studies of Science* 44 (2): 165–193.

Perkel, J. 2017. "How to Hack the Genome." *Nature* 547 (7664): 477–479.

Pretorius, I., and J. Boeke. 2018. "Yeast 2.0 – Connecting the Dots in the Construction of the World's First Functional Synthetic Eukaryotic Genome." *FEMS Yeast Research* 18: 4. doi:10.1093/femsyr/foy032. foy032.

Richardson, S. 2011. *Computer Assisted Design for Synthetic Biology*. PhD thesis, Johns Hopkins University. https://pqdtopen.proquest.com/doc/1115971321.html?FMT=AI.

Richardson, S. 2017. "Principles of Genome Design." Presentation at SB7: The Seventh International Meeting on Synthetic Biology, National University of Singapore, Singapore, June 13–16 2017.

Richardson, S., L. Mitchell, G. Stracquadanio, K. Yang, J. S. Dymond, J. E. DiCarlo, D. Lee, et al. 2017. "Design of a Synthetic Yeast Genome." *Science* 355: 1040–1044.

Rouse, M. 2016. "Agnostic." *TechTarget* Whatis.com. http://whatis.techtarget.com/definition/agnostic.

Smith, H., C. Hutchinson, C. Pfannkoch, and C. Venter. 2003. "Generating a Synthetic Genome by Whole Genome Assembly: ϕx174 Bacteriophage From Synthetic Oligonucleotides." *PNAS* 100 (26): 15440–15445.

Szymanski, E. 2018a. "What is the Terroir of Synthetic Yeast?" *Environmental Humanities* 10 (1): 40–62.

Szymanski, E. 2018b. "Remaking Yeast: Metaphors as Scientific Tools in *S. Cerevisiae* 2.0." *BioSocieties*,.

Szymanski, E., and J. Calvert. 2018. "Designing with Living Systems in the Synthetic Yeast Project." *Nature Communications* 9: 2950. doi:10.1038/s41467-018-05332-z.

Temme, K., D. Zhaob, and C. Voigt. 2012. "Refactoring the Nitrogen Fixation Gene Cluster From *Klebsiella Oxytoca*." *Proceedings of the National Academy of Sciences* 109 (18): 7085–7090.

US Department of Health & Human Services. 2013. "Microorganisms and Microbial-Derived Ingredients Used in Food (Partial list)." US Food and Drug Administration. http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/MicroorganismsMicrobialDerivedIngredients/default.htm.

van Dooren, T., E. Kirksey, and E. Münster. 2016. "Multispecies Studies: Cultivating Arts of Attentiveness." *Environmental Humanities* 8 (1): 1–23.

Voigt, C. 2017. "Genetic Circuit Design." Presentation at SB7: The Seventh International Meeting on Synthetic Biology, National University of Singapore, Singapore, June 13–16 2017.

Walker, R. 2017. *De novo Biological Engineering of a tRNA Neochromosome in Yeast*. PhD thesis, University of Edinburgh. https://www.era.lib.ed.ac.uk/handle/1842/28921.

Weiss, R. 2009. "Synthetic Biology: What's in a Name?" *Nature Biotechnology* 27: 1071–1073.

Yong, E. 2014. "Synthetic Yeast Chromosome." *The Scientist*. March 27 2014. https://www.the-scientist.com/news-opinion/synthetic-yeast-chromosome-37754.

Yong, E. 2017. "Now That We Can Read Genomes, Can We Write Them?" *The Atlantic*. May 10, 2017. https://www.theatlantic.com/science/archive/2017/05/we-can-read-genomes-easily-now-what-about-writing-them/526086/.